Characterization of *Bacillus amyloliquefaciens* spores after thermal and pressure-assisted thermal processing by infrared microspectroscopy and multivariate analysis

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Abstract

Pressure-assisted thermal processing (PATP) utilizes combination of pressure (500- 800MPa) and heat (90-120°C) to inactivate bacterial spores but limited studies have been documented regarding its mechanism of inactivation. Fourier transform infrared (FT-IR) microspectroscopy was used to compare and contrast biochemical changes occurring in *Bacillus amyloliquefaciens* spores during thermal processing (TP: 105°C-0.1MPa) and PATP (105°C-600MPa). Spore crops were prepared using two different media (TSAYE and NAYE). Surviving spores were enumerated after incubation at 32°C up to 72 hours. Spectra were collected by attenuated total reflectance (ATR) in mid-infrared region (4000-700 cm-1). A SIMCA model (900-1800 cm-1) could differentiate pressure resistance/sensitivity of different spore crops before they were further treated. $D_{105°C\text{-}0.1MPa}$ of spores grown on TSAYE and NAYE were 24.21±0.25 and 36.79±1.34 min, whereas $D_{105°C\text{-}600MPa}$ were 1.32±0.38 and 1.65±0.21 min, respectively. Surviving spores after treatments could be precisely predicted by PLSR models with r-value >0.98. Changes in acidic proteins and dipicolinic acid (DPA) structure modification were primarily detected during TP and PATP. Bands associated to spore inactivation were identified as 1381, 1415, and 1442 cm^{-1}, indicating the contribution of carboxylate (COO^-) vibration of calcium dipicolinate (DPA-Ca^{2+}), the interaction of Ca^{2+} with COO^-, and pyridine ring vibration of DPA/acid peptides, respectively. PATP showed additional effects on amide bands (1570-1650 cm^{-1}) of protein. ATR-IR microspectroscopy is a powerful tool to discriminate the combined pressure-heat spore resistances and obtain further information related to the contribution of DPA during spore inactivation by TP and PATP.

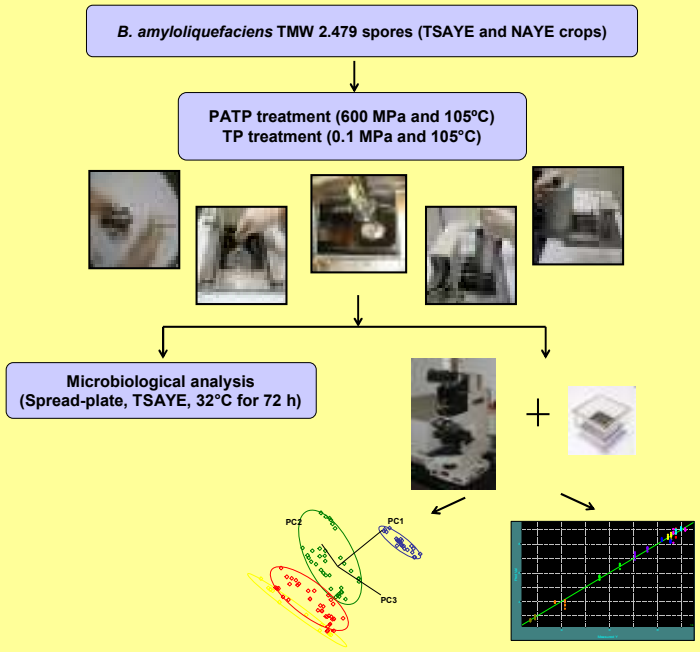
Introduction

- Pressure-assisted thermal processing (PATP) offers an alternative to preserve shelf-stable low-acid foods with minimal adverse impact on food quality.
  - Combination of elevated pressure (500-700 MPa) and temperature (90-121°C).
  - Rapid increase in temperature due to compression heating, and expansion cooling upon depressurization.
  - Potentially useful for preserving heat-sensitive shelf-stable products such as coffee, tea, soups, sauces, mashed potatoes, and egg products (Rajan *et al.*, 2006).
- Limited information is available on mechanism of spore inactivation by PATP.
  - At 50-500 MPa and 25-60ºC, spore germination by activating nutrient germinant receptors (Paidhungat *et al.*, 2002).
  - At 500-600 MPa and <60ºC, release of dipicolinic acid (DPA) from spore core through specific channels in the inner membrane or on the membrane itself (Black *et al.*, 2007).
- FT-IR spectroscopy has been shown as a promising technique to characterize biochemical changes during food processing (Subramanian et al., 2007).

Objectives

- To compare and contrast biochemical changes occurring in *B. amyloliquefaciens* TMW 2.479 spores during thermal processing (TP) and pressure-assisted thermal processing (PATP) by using FT-IR microspectroscopy.

Materials and Methods



Results and Discussion

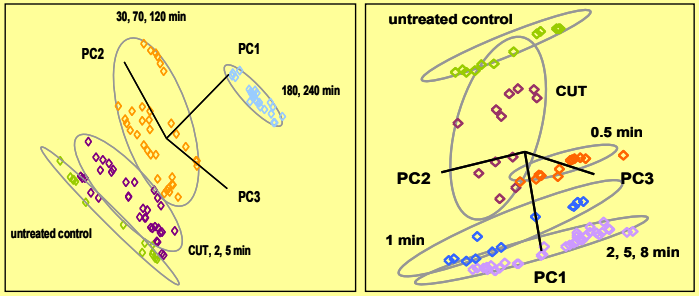


Figure 1 SIMCA class projection of *B. amyloliquefaciens* TMW 2.479 spores grown on NAYE after TP at 105°C-0.1 MPa (A) and PATP at 600 MPa-105°C (B)

- Simple, rapid, and efficient protocol was developed by combining hydrophobic grid membrane filtration and infrared microspectroscopy.
- Three distinctive groups were observed during TP, whereas there was subtle differences in spore structures among 2, 5, and 8 min PATP treated samples.
- Surviving spore populations from standard plate count could be well-correlated with spectra region of 900-1800 cm^{-1}.

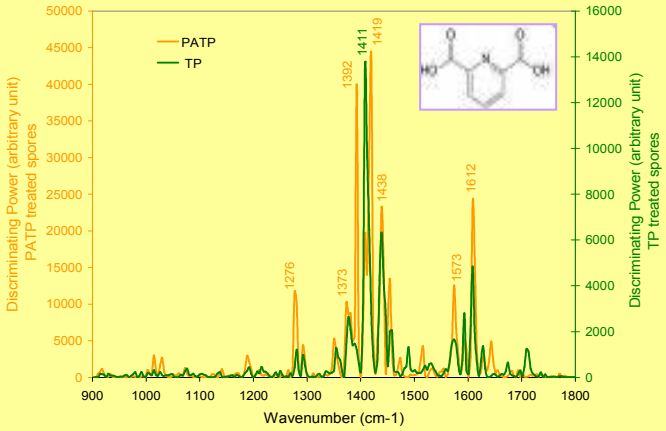


Figure 2 Comparison of discriminating power plot of *B. amyloliquefaciens* TMW 2.479 spores grown on NAYE after TP (105°C, 0.1 MPa, 120 min) and PATP (600 MPa, 105°C, 5 min) treatments resulting in 3 log reduction

- Irrespective to treatments, three bands associated to spore inactivation were identified as 1381, 1415, and 1442 cm^{-1} indicating contribution of COO^- vibration of DPA-Ca^{2+}, interaction of Ca^{2+} with COO^-, and pyridine ring vibration of DPA, respectively.
- PATP treatment also modified amide region (1570-1650 cm^{-1}), suggesting the action toward spore coats

Conclusions

- Release of DPA serves as a key component triggering spore inactivation during TP and PATP.
- PATP accelerated spore inactivation by acting upon specific targets of DPA and/or acidic protein structures.
- ATR-IR microspectroscopy combined with multivariate analysis is a power tool to discriminate the combined pressure-heat spore resistance and monitor biochemical changes associated to spore inactivation during TP and PATP.

References

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